PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	04.04.2006
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
4843PTWO/AG/la	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/050728	18.02.2005	19.02.2004

Applicant
INSTITUTO NAZIONALE DELLE MALATTIE INFETTIVE ..

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4843PTWO/AG/a	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2005/050728	International filing date *(day/month/year)* 18.02.2005	Priority date *(day/month/year)* 19.02.2004
International Patent Classification (IPC) or national classification and IPC INV. G01N33/569 C07K14/35		
Applicant INSTITUTO NAZIONALE DELLE MALATTIE INFETTIVE ..		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.02.2006	04.04.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Gundlach, B Telephone No. +31 70 340-4478

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2005/050728

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-47 as originally filed

Sequence listings part of the description, Pages

1 as originally filed

Claims, Numbers

1-36 received on 23.02.2006 with letter of 17.02.2006

Drawings, Sheets

1-10 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 22-24

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 22-24

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21,25-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	6-18,25-27
	No:	Claims	1-5,19-21,28-36
Industrial applicability (IA)	Yes:	Claims	1-21,25-36
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III

Claims 22 to 24 were found to lack technical features. The resulting unclarity was such that no meaningful search could be carried out for these claims. As no search has been done for these claims they will also not be examined (Rule 66.1(e) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1: WO 2004/005925 A (ISIS INNOVATION LIMITED; LALVANI, AJIT; EWER, KATIE) 15 January 2004 (2004-01-15)
D2: WO 02/054072 A (ISIS INNOVATION LIMITED; LALVANI, AJIT) 11 July 2002 (2002-07-11)
D3: WO 99/04005 A (INSTITUT PASTEUR ET AL.) 28 January 1999 (1999-01-28)
D4: WO 98/16646 A (CORIXA CORPORATION) 23 April 1998 (1998-04-23)

2 Novelty

Subject-matter of claims 1 to 21 and 25 to 36 is novel since the peptides/nucleic acids of SEQ ID NOS: 1 to 8 are novel in light of the prior art cited.

3 Inventive step

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 to 5, 19 to 21 and 28 to 36 does not involve an inventive step in the sense of Article 33(3) PCT.

3.1 D1 is regarded as being the closest prior art to the subject-matter of claim 3, and discloses (the references in parentheses applying to this document):

An epitope of *M. tuberculosis* represented by a peptide (claims 14, 15 and 16, peptides 21, GNFERISGDLKTQID). The peptide of SEQ ID NO: 2 in claim 3 differs

therefrom in that it is 2 amino acids shorter on the N-terminal side and 1 amino acid longer on the C-terminal side. There is no technical effect derivable from this difference. The objective problem to be solved can therefore be formulated as relating to: How to provide a further peptide relating to the same epitope? The solution, a peptide according to SEQ ID NO: 2 is considered not to involve an inventive step for the following reasons: A person skilled in the art would know that a peptide representing an epitope can be varied in length without loss of the immunologic activity. Accordingly, it would be obvious for a person skilled in the art to come-up with the peptide represented by SEQ ID NO:2 or its corresponding nucleic acid sequence as in claim 33.

In that respect it should be remarked that products like compositions, peptides, kits, etc. are "entities". For entities the term "for use" has to be interpreted as "**suitable** for use".

3.2 D1 furthermore discloses

- a peptide representing the same epitope as peptide of SEQ ID NO:8 (peptide 32, EISTNIRQAGVQYSR)
- a composition for use in medicine comprising at least a peptide sequence of SEQ ID NO:2 as in claim 1
- the epitopes represented by the peptides according to SEQ ID NO:10 and 12 as in claim 2 (claim 16, peptide 2, WNFAGIEAAASAIQG and peptide 14, NNALQNLARTISEAG)
- use of peptides and a diagnostic kit as in claims 28 to 30 (cl. 92 to 94).

3.3 For subject-matter of claim 4 D1 is considered to represent the closest prior art and discloses:
An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis (see D1, claims 1 and 16). Subject-matter of claim 4 differs from D1 in that variations of the peptides mentioned in 3.2 above are being used. The alleged technical effect of this difference is that a discrimination between active tuberculosis, latent and recent infection in a single step can be achieved. The objective problem to be solved would thus be: How to provide a method which allows discrimination between active tuberculosis, latent and recent infection in a single step? The solution,

use of slightly varied peptides of D1 cannot be considered to represent an inventive step for the following reasons: No data is shown that would hint at the fact that the peptides of the present application (esp. SEQ ID NO: 2, 8, 10 and 12) would perform any different from the peptides of D1. Indeed either claims 4 and 5 lack essential features which are necessary to solve the underlying technical problem or the problem to be solved has to be formulated in a broader way: How to provide a further method of diagnosing tuberculosis? The solution provided by claims 4 and 5 would be not inventive in light of the disclosure of D1.

3.4 For subject-matter of claim 6 D1 is considered to represent the closest prior art and discloses a method as already discussed under 3.3 above. Subject-matter of claim 6 differs from D1 in that specific combinations of peptides are used. The technical effect of this difference is that it allows a single step discrimination between active tuberculosis, latent and recent infection. The objective problem to be solved can therefore be formulated as relating to: How to provide a method which allows discrimination between active tuberculosis, latent and recent infection in a single step? The solution, the combination of peptides disclosed in claim 6 are neither disclosed nor suggested in the prior art at hand.

Accordingly, subject-matter of claim 6 and dependent claims 7 to 18 are considered to involve an inventive step. Subject-matter of claims 25 to 27 is considered to be inventive as they relate to a kit specially adapted to the methods above or the use of peptides to produce such a kit.

3.5 Claims 19 to 21 do not contain any **technical** features which could render their subject-matter inventive.

3.6 The analysis in item 3.1 for the peptides of claim 3 could also be done in light of the following documents which also disclose peptides representing the same epitopes:
- in as far as SEQ ID NO: 2 and 1 are concerned: D3 (see claim 21, SEQ ID NO:8)
- in as far as SEQ ID NO: 4, 6, 3 and 5 are concerned: D4 (see page 122, SEQ ID NO:95 and 96).

4 Industrial Applicability

4.1 Claims 1 to 21 and 25 to 36 are industrially applicable.

EPO - DG 1

23. 02. 2006

NEW SET OF CLAIMS: (55)

1) A composition for use to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, said composition comprising at least a peptide sequence selected from the group of SEQ. ID No 2, No 4, No 6 and No 8 and corresponding mixtures.

2) A composition according to claim 1 further comprising at least a peptide sequence selected in the group of SEQ. ID No 10 and No 12 and corresponding mixtures.

3) A peptide sequence selected from the group consisting of: SEQ. ID No. 2, 4, 6, 8.

4) An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, whereby an aliquot of whole venous blood or PBMC (peripheral blood mononuclear cells) is admixed with an effective amount of the composition according to claim 1.

5) An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, whereby an aliquot of whole venous blood or PBMC (peripheral blood mononuclear cells) is admixed with an effective amount of the composition according to claim 2.

6) An *in vitro* method to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, said method comprising the following steps:

a) admixing an aliquot of venous blood or mononuclear cells (PBMC) isolated from venous blood with a mixture comprising each of the following reagents:

- Reagent 2, at least one intact protein selected in the group of ESAT-6 and CFP-10, and corresponding mixtures;

- Reagent 3: at least one ESAT-6 peptide selected in the group of SEQ ID NO 10, 12, and corresponding mixtures, diluted in a solvent
- Reagent 4: at least one CFP-10 peptide selected in the group of SEQ ID NO 2, 4, 6, 8, and corresponding mixtures, diluted in a solvent;
- Reagent 5: a mixture of at least one ESAT-6 and CFP-10 peptides, selected in the groups of SEQ ID NO 10, 12 and SEQ ID NO 2, 4, 6, 8, and mixtures thereof, diluted in a solvent;

b) measuring T-lymphocytes response.

7) A method according to claim 6 wherein the mixture in step a) further comprises:

- Reagent 6: an aspecific T-Lymphocyte stimulus, as phythoemoagglutinine (PHA), positive control
- Reagent 7: PPD, Purified Protein Derivative.

8) A method according to claims 6-7 wherein the mixture in step a) further comprises:

- Reagent 1: CTR, complete culture medium or medium comprising the solvent concentration present in Reagents 3-5 (negative control).

9) A method according to claim 8 wherein the solvent is dimethyl sulfoxide (DMSO).

10) A method according to claims 6-9, whereby T-lymphocytes response is measured by: ELISPOT, FACS, whole blood ELISA.

11) A method according to claim 10 wherein said cytokine is selected from the group consisting of: IFN-gamma, TNF-alpha, GMSF, interleukins IL1-IL24.

12) A method according to claims 6-11 wherein the response is mediated by CD4 T lymphocytes .

13) A method according to claims 6-12 wherein, in case whole venous blood is used, said blood is placed into heparinised test tubes, and T-lymphocyte response is assessed by ELISA on plasma.

14) A method according to claims 6-12 wherein, in case PBMC are used, T-lymphocyte response is assessed by ELISPOT or Flow Cytometric Analysis.

15) A method according to claims 6-12 wherein PBMC are obtained from whole blood by density gradient centrifugation using a method based on the use of filter-equipped tubes for separation of leukocytes.

16) A method according to claims 6-12 wherein the incubation is carried out on PBMC from whole blood for at least 40 hours with subsequent quantitative determination of IFN-gamma production by Antigen-Specific T lymphocytes by the ELISPOT method.

17) A method according to claims 6-12 wherein the incubation of PBMC from whole blood is carried out for at least 16 hours with subsequent determination of IFN-gamma production by Antigen-Specific T lymphocytes, said determination being both qualitative in terms of presence/absence of Antigen-Specific T lymphocytes, by FACS, and quantitative in terms of percentage and frequency of specific cells per mm^3 of blood.

18) A method according to claims 6-12 wherein the incubation is performed on whole blood for approximately 24 hours with subsequent quantitative determination of IFN-gamma production by Antigen-Specific T lymphocytes by ELISA.

19) A method to elaborate results from output values from method according to claim 6-18, comprising the following steps:

- Calculate the absolute values, from subtracting the output value of the negative control, sample admixed with Reagent 1, from the output values for the reagents R2-R7
- Compare said absolute values with the correspondent cut-off values, and if:
    - below said value, the output is not valid.
    - above said value, determine if it fulfils the following criteria: value for Reagents 2, 6, 7 is at least 3-fold higher than value for Reagent 1; value for Reagent 3 is at least 2-fold higher than value for Reagent 1; value for Reagents 4 and 5 is at least 4-fold higher than value for Reagent 1;
- Ascertain if the response for Reagent 6 is positive: if not, the patient is diagnosed anergic, and the assay is not further evaluable; if the response for Reagent 6 is positive :
- Ascertain if the response for Reagent 7 is positive: if not, the patient is diagnosed as a healthy subject, if so
- Ascertain if the response for Reagent 2 is positive: if not, the patient is diagnosed as BCG-vaccinated or exposed to atypical *Mycobacteria,* if so
- Ascertain if the response to Reagent 3 or 4 or 5 or a mixture of these is also positive: if not, the patient is diagnosed as a latent TB patient or a TB patient under or after efficacious anti-TB therapy; if the response to Reagent 3 or 4 or 5 or a mixture of these is positive, the patient is diagnosed as an active TB disease patient or a patient recently infected or re-infected with *M. tuberculosis*

20) A method according to claim 19, where the cut-off minimum is 34 SFCs reading for Reagent 3, 4 and 5 ELISPOT output, 36 SFCs reading for Reagent 2 ELISPOT output, 60 SFCs reading for Reagent 6, 7.

21) A method to elaborate a diagnosis according to claim 19, where the cut-off minimum is 0.6 IU/mL for Reagents 2-7.

22) A system to elaborate results from output values from method according to claim 6-18 characterised in that it comprises means for performing the steps of the method of any of the claims from 19 to 21.

23) A computer program comprising computer program code means adapted to perform all the steps of claim 19-21 when said program is run on a computer.

24) A computer readable medium having a program recorded thereon, said computer readable medium comprising computer program code means adapted to perform all the steps of claim 19-21 when said program is run on a computer

25) A diagnostic kit to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*, , comprising:

- Reagent 1: CTR, complete culture medium or medium comprising the solvent concentration present in Reagents 3-5;
- Reagent 2, at least one intact protein selected in the group of ESAT-6 and CFP-10, and corresponding mixtures;
- Reagent 3: at least one ESAT-6 peptide selected in the group of SEQ ID NO 10, 12, and corresponding mixtures, diluted in a solvent ;
- Reagent 4: the composition according to claim 1 diluted in a solvent;
- Reagent 5: the composition according to claim 2 diluted in a solvent;

- Laboratory materials and instructions for test procedure.

26) A kit according to claim 25 that further comprises:

- Reagent 6: an aspecific T-Lymphocyte stimulus, as PHA, phythoemoagglutinine;
- Reagent 7: PPD, Purified Protein Derivative.

27) Use of a peptide selected in the group of SEQ ID NO 2, 4, 6 and 8 and corresponding mixtures in producing the kit according to claims 25 or 26.

28) Use of a peptide sequences selected in the group of SEQ. ID No.2, 4, 6, 8 and corresponding mixtures in combination with a peptide selected in the group of SEQ ID NO 10, 12 and corresponding mixtures in producing a diagnostic kit to diagnose and monitor in a single step different states of tuberculosis for the discrimination of active tuberculosis, latent infection and recent infection with *M. tuberculosis*.

29) Use of a kit according to claims 27-28, wherein the subject to be tested is an individual selected among mammals, such as a primate, cow, sheep, pig, badger or rodent, e.g. a mouse or rat, humans being included.

30) Use of a kit according to claims 27-28 wherein the subjects to be tested are subjects at risk of tuberculosis.

31) Use of a kit according to claims 27-28 wherein the subjects to be tested are children, health care workers and immuno-compromised patients.

32) Nucleotide sequence encoding the peptides according to claim 3.

33) Nucleotide sequence: SEQ ID NO 1.

34) Nucleotide sequence: SEQ ID NO 3.

35) Nucleotide sequence: SEQ ID NO 5.

36) Nucleotide sequence: SEQ ID NO 7.